Characterization of H_2O_2 resistant microbes isolated from spacecraft assembly facility

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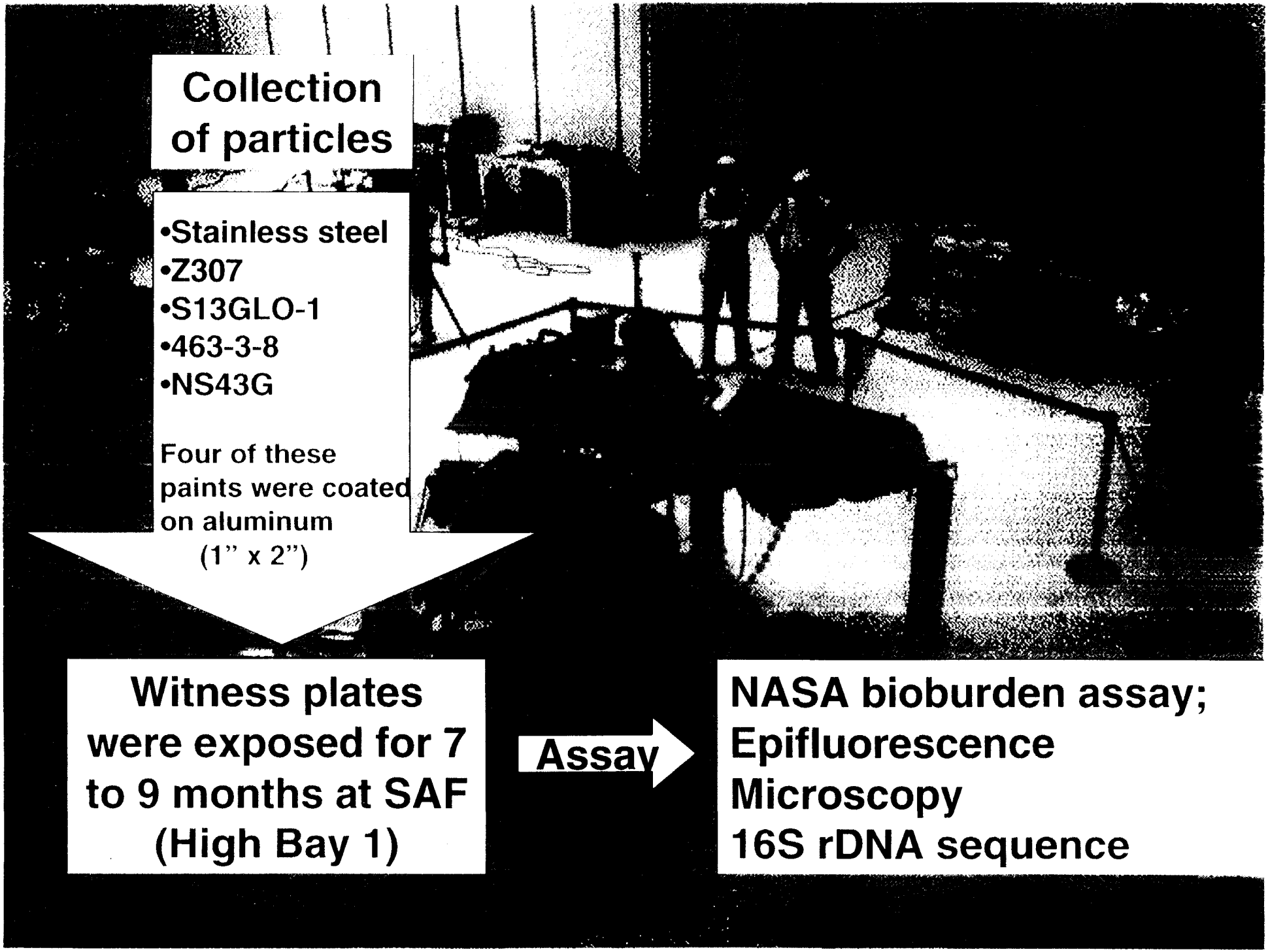
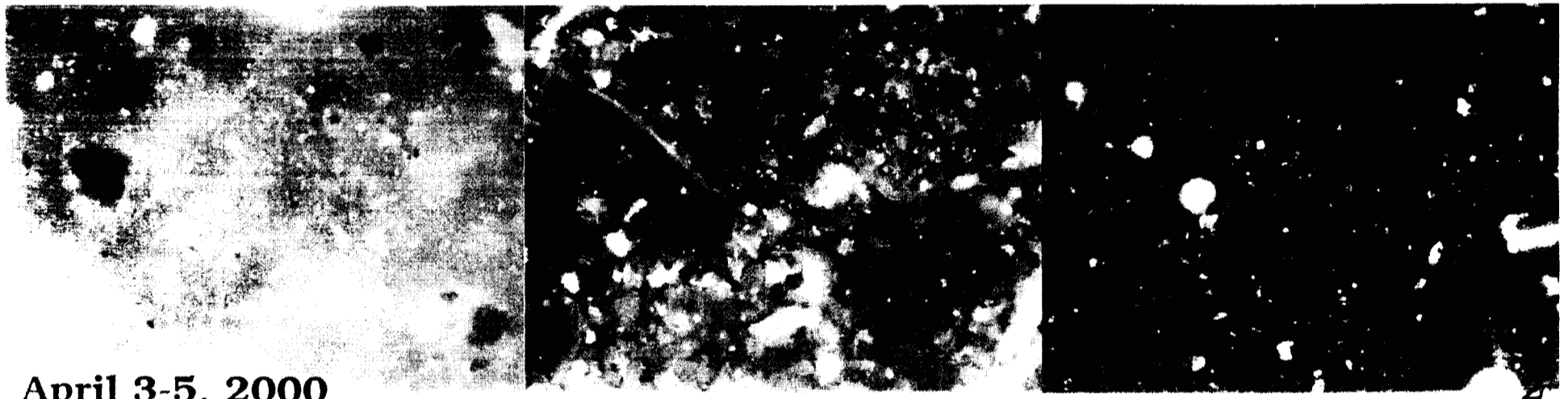

Collection of particles
•Stainless steel
•Z307
•S13GLO-1
•463-3-8
•NS43G
Four of these paints were coated on aluminum (1" x 2")
Witness plates were exposed for 7 to 9 months at SAF (High Bay 1)
Assay
NASA bioburden assay;
Epifluorescence Microscopy
16S rDNA sequence

Microscopic images of witness plates

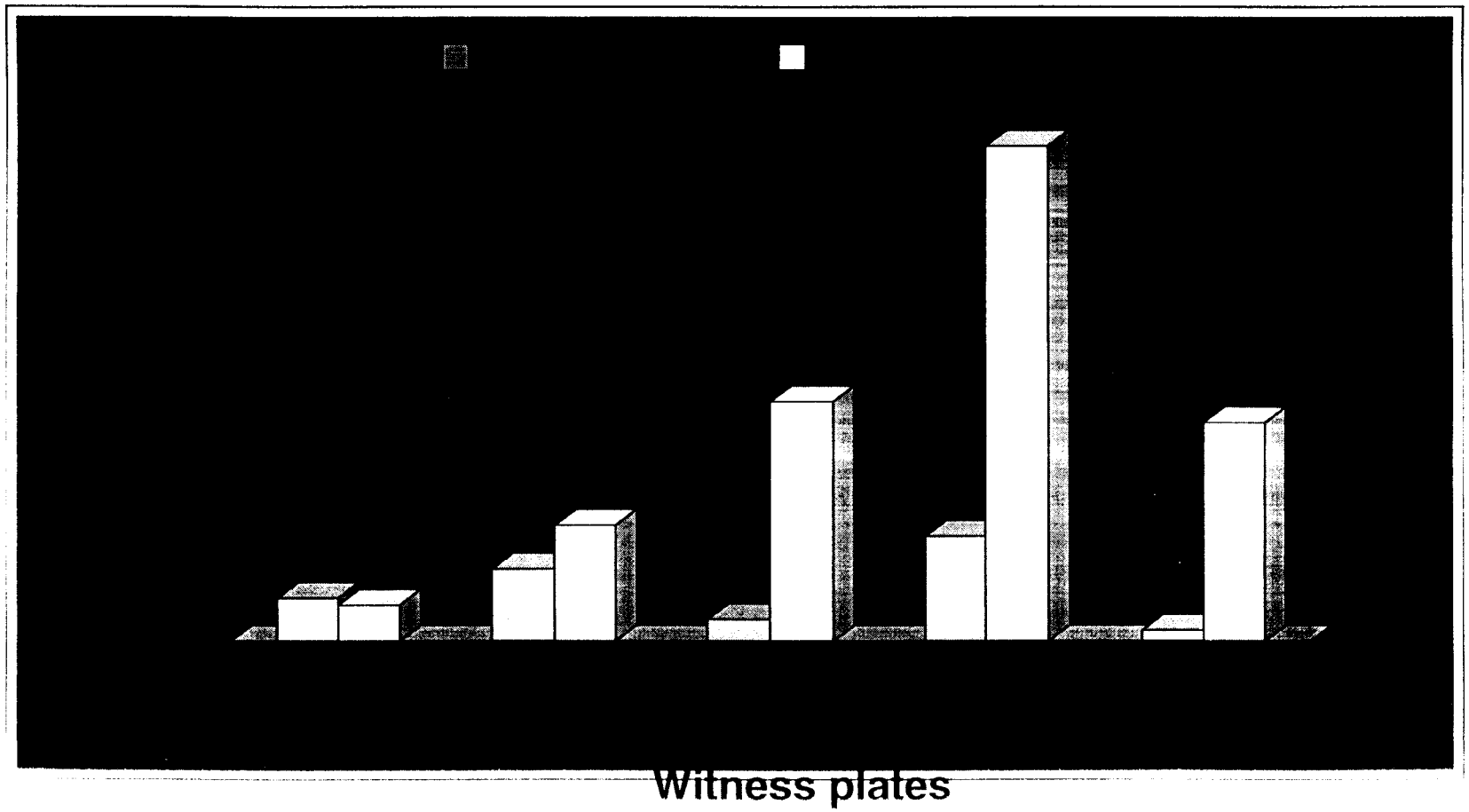
Stainless steel Z-307

- Hard to differentiate microbes from particles.
- NS43G paint attracts more particles.

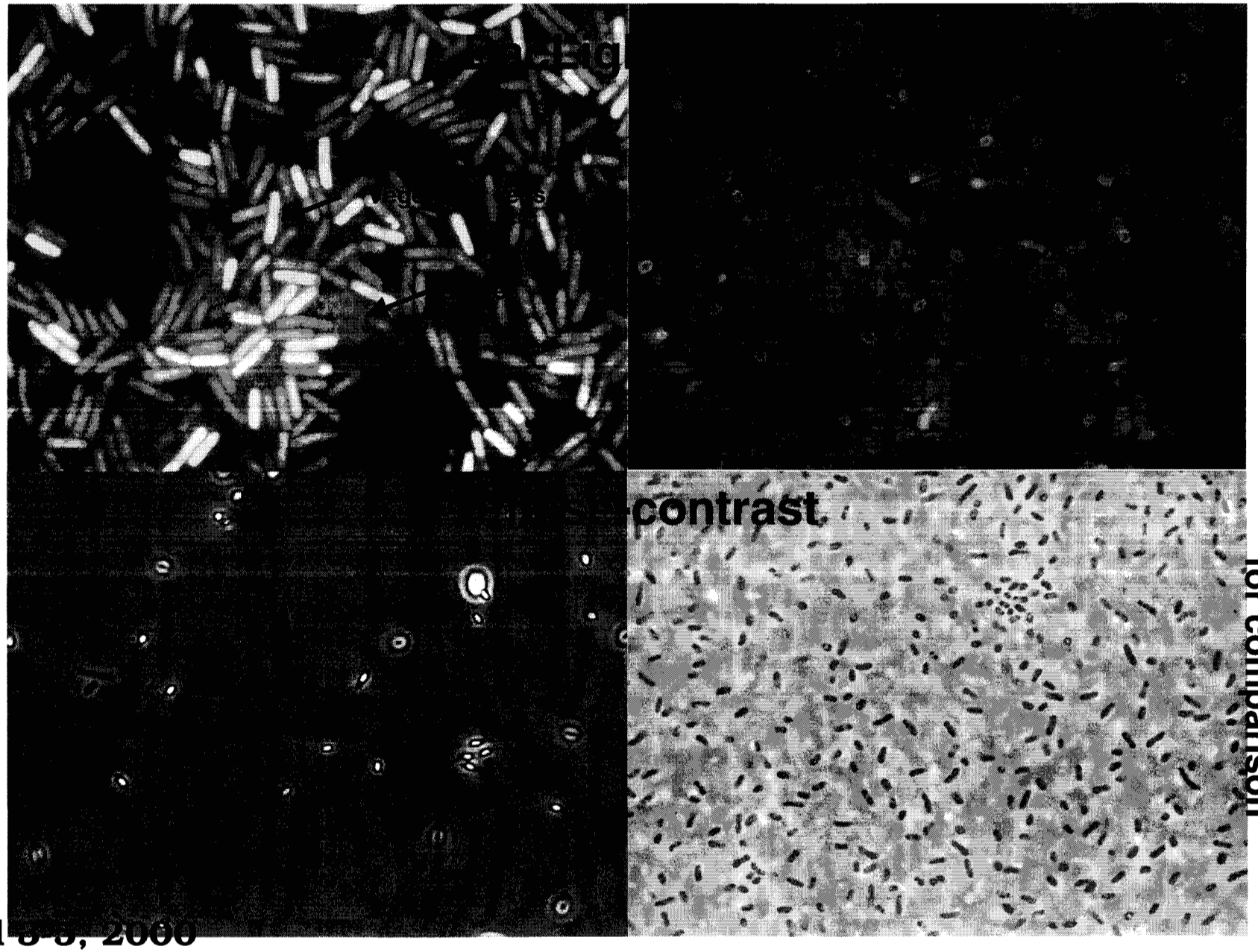
S13GLO-1 NS43G (10x) 463-3-8



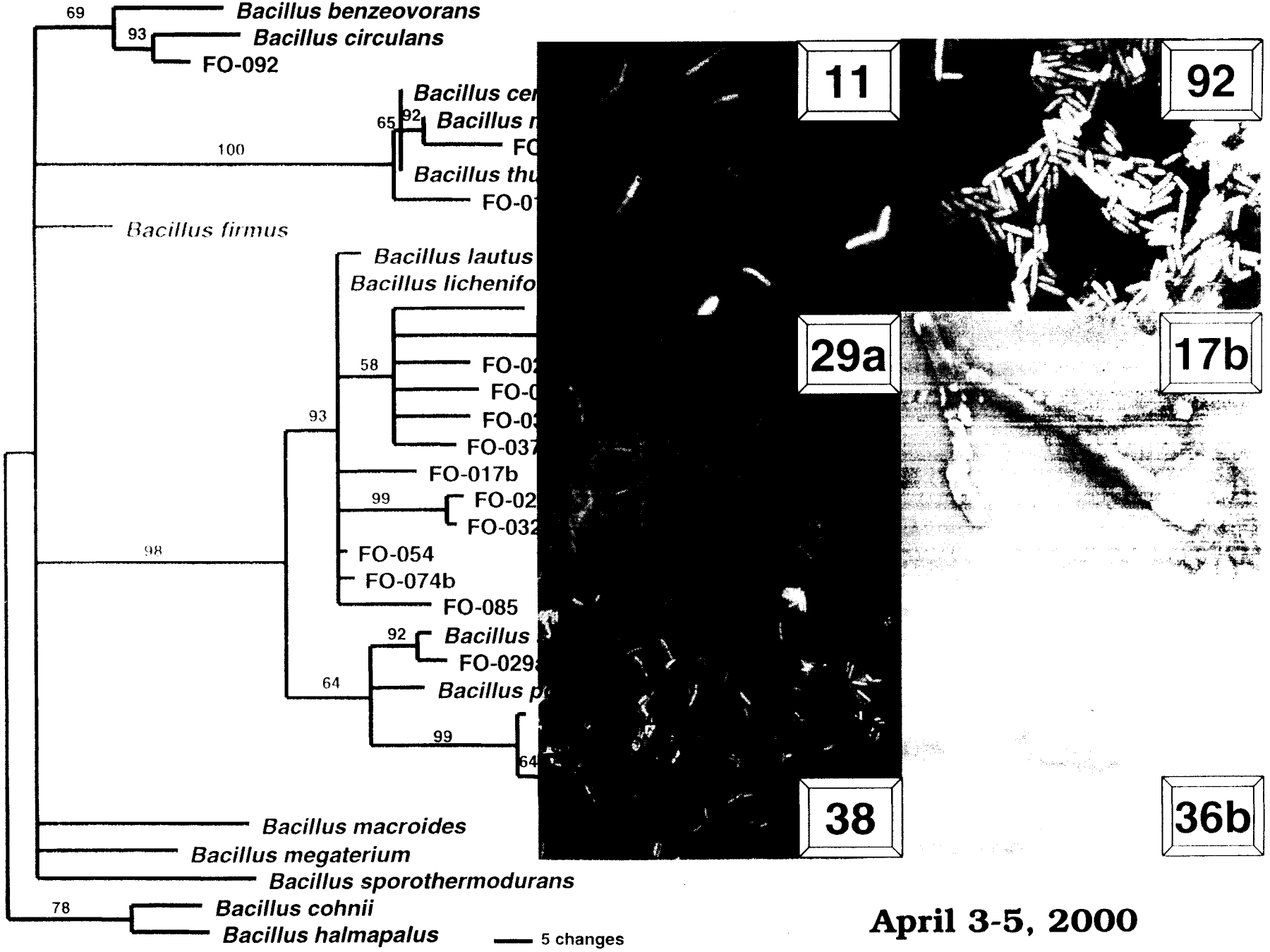
NASA Bioburden Assay



A novel *Bacillus* species that produce large spore



Phylogeny of *Bacillus* species isolated from SAF

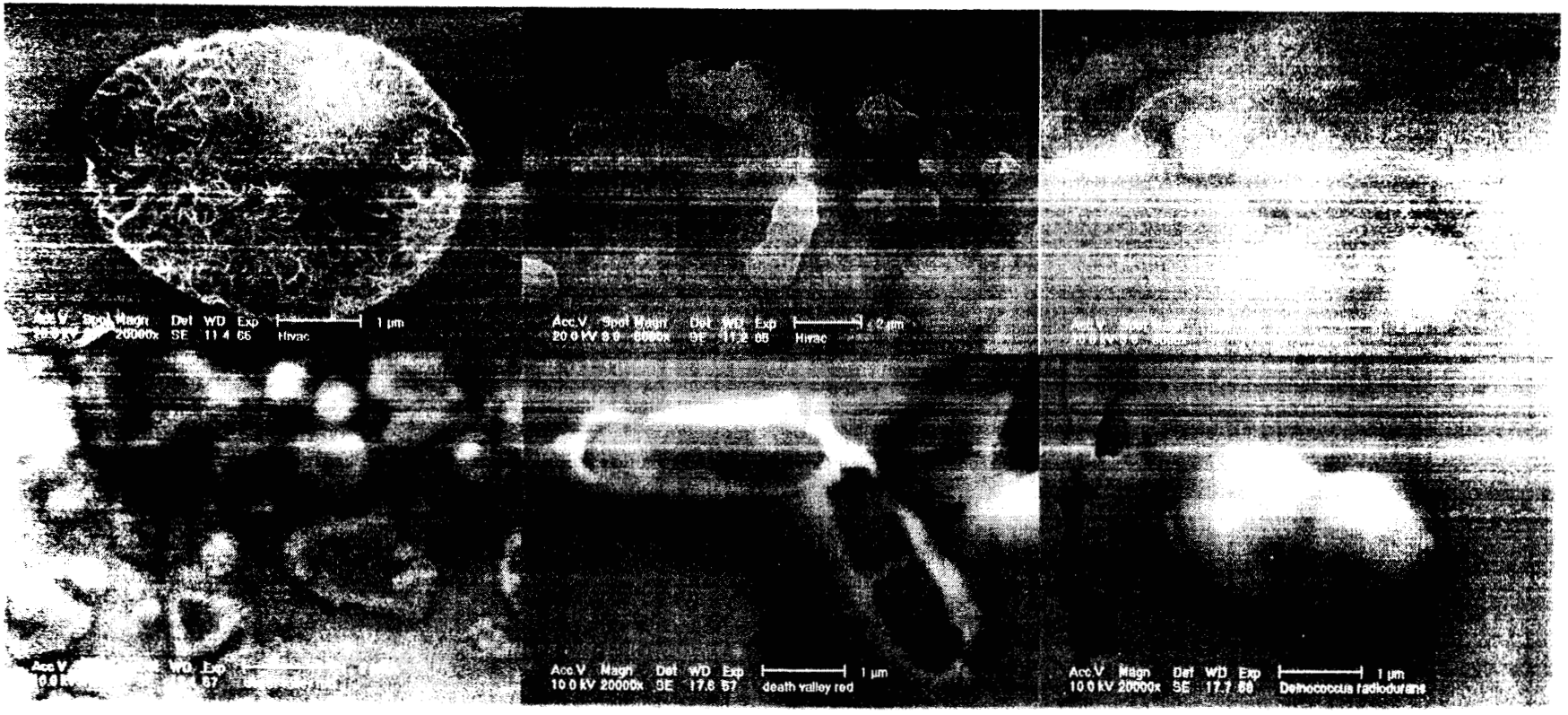


Are SAF conditions extreme?

- About 44% of 39 strains isolated as per NASA heat shock procedure (85°C for 15-min) and 10% of 40 strains isolated as per NASA standard assay (grown in TSA at 32 °C) showed growth at 60°C.
- 50% of 89 strains showed growth at high salt concentration (10% NaCl conc.)
- Majority of the cultivable microbes were Gram positives and mainly spore-formers.
- 5% are coccoid.
- 16S rDNA sequences revealed that majority of the bacterial species are *Bacillus.*
- Uncharacterized *Bacillus* species are recognized.

Morphological changes after H_2O_2 sterilization in Gram-positive microbes

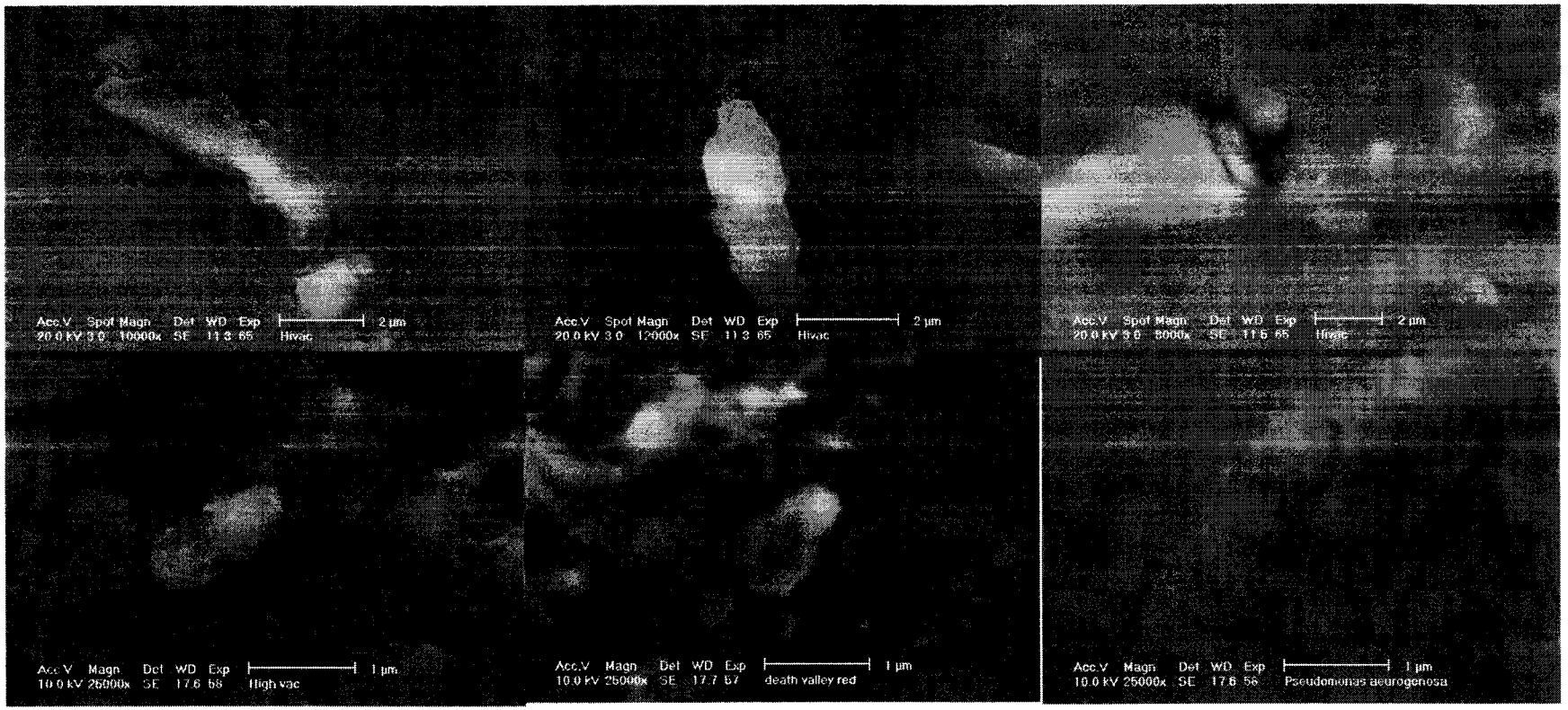
Bacillus megaterium ***Bacillus subtilis*** ***Deinococcus radiodurans***



All Gram-positive species tested were susceptible to H_2O_2 (3.6 mg/L)

Morphological changes after H_2O_2 sterilization in Gram-negative microbes

Escherichia coli | *Citrobacter freundii* | *Pseudomonas aeruginosa*



After 4-inj. Before

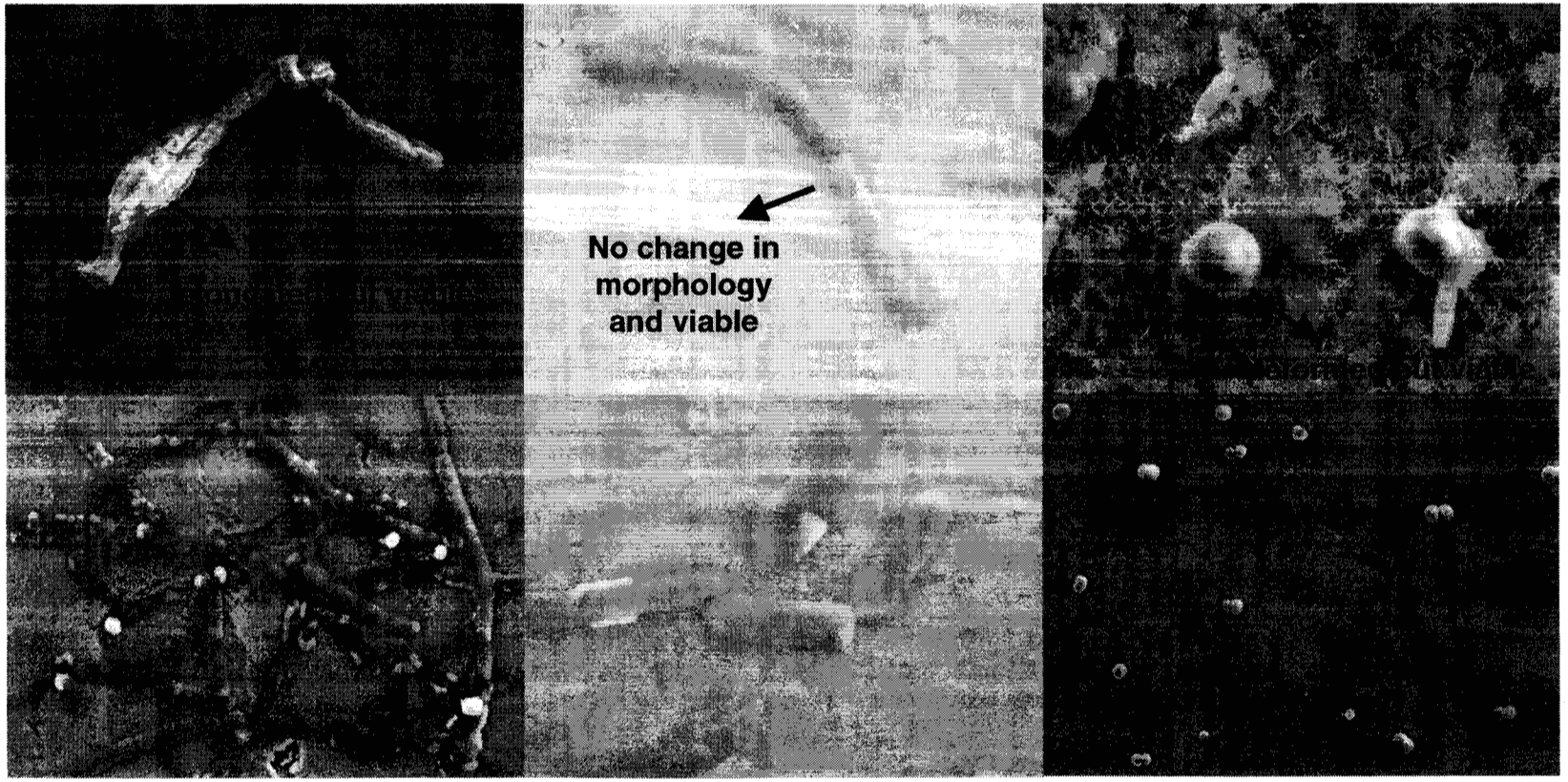
All Gram-negative species tested were susceptible to H_2O_2 (3.6 mg/L)

Morphological changes after H_2O_2 sterilization in microbes isolated from SAF

Bacillus licheniformis FO-17b

Bacillus pumilus FO-36b

Staphylococcus capitis FO-36a



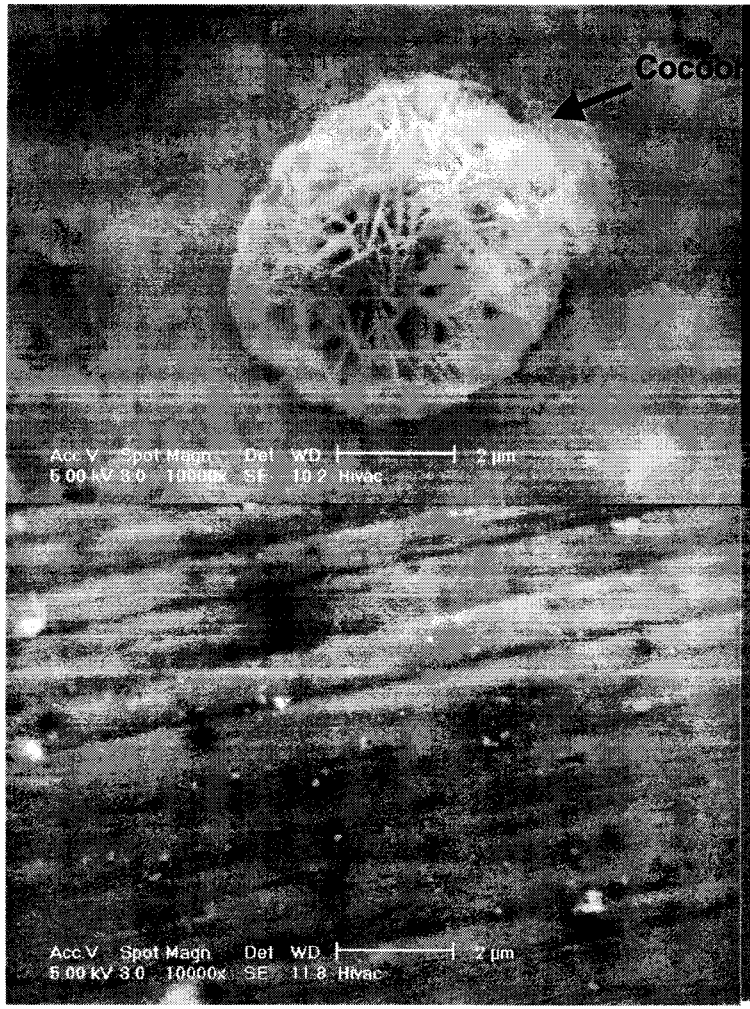
~30% of the SAF microbes were resistant to various doses of H_2O_2 (4~16 mg/L)

Oxidation of aluminum 6061 by various sterilization processes

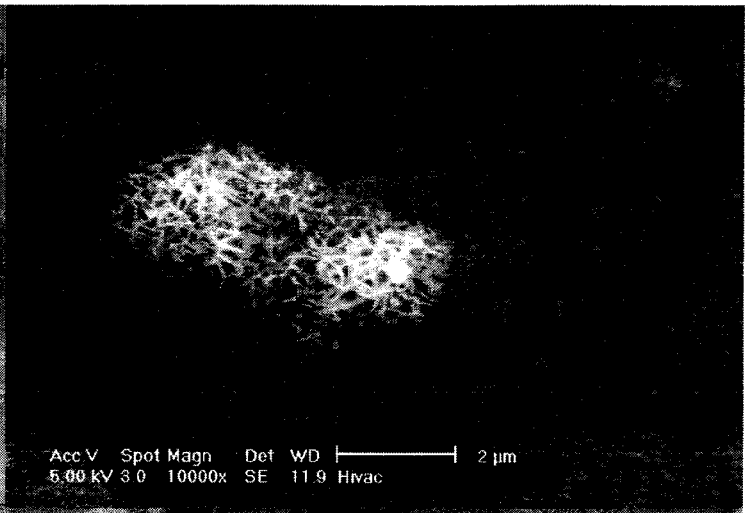
More Aluminum oxide layers in the order of 500°C > H_2O_2 > Autoclaving > Untreated

Effect of autoclaving in the adhesion of microbial species on aluminum 6061

Spore-spiked 18-rms polish; Autoclaved;



Spore-spiked mirror polish; Autoclaved;



Spore-spiked mirror polish; non-autoclaved;

B. subtilis cells; mirror polish; non-autoclaved

Microbes tend to adhere onto Aluminum surfaces once oxide layers were made available

Conclusion

- The atmosphere of a spacecraft assembly facility should be considered as extreme environment. Many strains isolated from the JPL-SAF were sopore-formers and exhibited intense growth at 60°C, and at 10% NaCl. Majority of these cultivable isolates were identified as *Bacillus* species.
- 16S ribosomal RNA sequence analysis revealed existence of *Bacillus licheniformis, B. pumilus, B. cereus, B. subtilis, Staphylococcus capitis, Planococcus citreus* and *Micrococcus lylae*.
- A novel uncharacterized *Bacillus* sp. that produce unusually big spores was isolated.
- Eight strains exhibited resistance to various doses of H_2O_2 vapor.
  - *B. licheniformis* (4 strains); *B. pumilus* (3 strains); *S. capitis* (1 strain).
  - Representatives of various microbes procured from culture collection were susceptible where as 30% of the wild strains isolated from SAF showed resistance to H_2O_2 vapor.
- Isolation of microbes that are resistant to H_2O_2 vapor has significant implications for the quality of products in the pharmaceutical and spacecraft industries that depend on low-heat sterilization technology.